https://doi.org/10.15360/1813-9779-2024-1-2351

The Effect of High Nitric Oxide Concentrations on Oxygenators in Cardiopulmonary Bypass Machines (Experimental Study)

Alexey M. Radovskiy[1]*, Ilya V. Vorotyntsev[2], Artem A. Atlaskin[2], Anton N. Petukhov[2], Sergey S. Kryuchkov[2], Maria E. Atlaskina[2], Anna N. Stepakova[2], Alexander O. Marichev[1], Egor K. Barygin[1], Victor V. Osovskikh[1], Victor D. Selemir[3], Sergey N. Buranov[3], Vladimir V. Golovanov[3], Alexander S. Shirshin[3], Yulia V. Valueva[3], Vladimir V. Pichugin[4], Stepan E. Domnin[5], Andrey E. Bautin[1]*

[1] Almazov National Medical Research Center,
2 Akkuratova str., Saint-Petersburg 197341, Russia
[2] Mendeleev University of Chemical Technology of Russia,
9 Miusskaya sq., Moscow 125047, Russia
[3] Russian Federal Nuclear Center, All-Russian Scientific Research Institute of Experimental Physics,
37 Mira av., Sarov 607188, Russia
[4] Privolzhsky Research Medical University,
10/1, Minin and Pozharsky square, Nizhniy Novgorod 603005, Russia
[5] Research Institute «Specialized Cardiosurgical Clinical Hospital named after Academician B. A. Koroleva»,
209 Vaneeva street, Nizhny Novgorod 603950, Russia

For citation: *Alexey M. Radovskiy, Ilya V. Vorotyntsev, Artem A. Atlaskin, Anton N. Petukhov, Sergey S. Kryuchkov, Maria E. Atlaskina, Anna N. Stepakova, Alexander O. Marichev, Egor K. Barygin, Victor V. Osovskikh, Victor D. Selemir, Sergey N. Buranov, Vladimir V. Golovanov, Alexander S. Shirshin, Yulia V. Valueva, Vladimir V. Pichugin, Stepan E. Domnin, Andrey E. Bautin.* The Effect of High Nitric Oxide Concentrations on Oxygenators in Cardiopulmonary Bypass Machines. *Obshchaya Reanimatologiya = General Reanimatology.* 2024; 20 (1): 50–62. https://doi.org/10.15360/1813-9779-2024-1-2351 [In Russ. and Engl.]

***Correspondence to:** Alexey M. Radovskiy, svetlbii@mail.ru; Andrey E. Bautin, abautin@mail.ru

Summary

The aim of the study. To study the effect of high nitric oxide concentrations on hollow polypropylene fibers of oxygenators.

Materials and methods. The study was conducted in two stages. At the first stage, we evaluated the stability of oxygenator membrane made of hollow polypropylene fibers after six hours of exposure to air-oxygen mixture containing NO at 500 parts per million, or 500 pro pro mille (ppm) concentration, using mass spectrometry and infrared spectroscopy. At the second stage, an experiment with cardiopulmonary bypass (CPB) was conducted on 10 pigs. In the study group (*N*=5) animals sweep gas was supplied to the oxygenator as an air-oxygen mixture with NO at 100 ppm. In the control group animals (*N*=5) an air-oxygen mixture was used without NO. The CPB lasted for 4 hours, followed by observation for 12 hours. NO, NO_2 (at the inlet and outlet of the oxygenator), and the dynamics of methemoglobin were evaluated. After weaning of animals from CPB, the oxygenators were tested for leakproofness, and scanning electron microscopy (SEM) was performed.

Results. The oxygenator made of polypropylene hollow fibers retained its gas transfer parameters after six hours of exposure to air-oxygen mixture containing NO at 500 ppm. Based on IR-Fourier spectroscopy findings, NO did not affect structural integrity of polypropylene membranes. NO added to gas mixture at 100 ppm did not increase NO_2 to toxic level of 2 ppm in 91% of control tests during 4 hours CPB in pigs; mean value was 1.58±0.28 ppm. Methemoglobin concentration did not exceed the upper limit of permissible level (3%), and there were no statistically significant differences with the control group. All tested oxygenators have passed the leakproofness test. According to SEM findings, larger amounts of fibrin deposits were found in the control group oxygenators vs study group.

Conclusion. There were no negative effects of NO at 500 ppm concentration on the oxygenator membrane made of hollow polypropylene fibers. NO at 100 ppm in a gas-mixture supplied to oxygenators did not lead to an exceedance of safe NO_2 and methemoglobin concentrations in an animal model. Reduced fibrin deposits on hollow fibers of polypropylene oxygenator membranes were observed when with NO at a level of 100 ppm was added to a gas mixture.

Keywords: nitric oxide; cardio-pulmonary bypass; oxygenator; polypropylene hollow fibers; cardiac surgery.

Conflict of interest. The authors declare no conflict of interest.

Funding and Support. The research was carried out with the support and in cooperation with the Russian Federal Nuclear Center, All-Russian Scientific Research Institute of Experimental Physics under the contract SD-22-04-48 for performance of a constituent part of the research. Part of this research was carried out within the framework of the State task No. 123021000129-1 implementation «Development of a new device for supplying nitric oxide synthesized from ambient air to heart-lung and auxiliary blood circulation devices».

Introduction

The vast majority of cardiac surgery is performed under cardiopulmonary bypass (CPB) [1]. Despite improvements in perfusion techniques and the development of safer equipment and supplies, CPB remains a non-physiologic procedure and has adverse effects on the human body. Negative effects of CPB include systemic inflammatory response syndrome, ischemia and reperfusion injury, and blood cell damage leading to hemolysis [2–4]. Cell-free hemoglobin (cfHb) resulting from hemolysis is removed from the circulation by haptoglobin, the CD163 protein. When the intravascular mechanisms for removing cfHb are exhausted, its level in the blood rises, with adverse clinical consequences. The heme formed during the degradation of cfHb is a cytotoxic pro-oxidant that catalyzes the formation of free radicals [5]. In addition, the negative effects of cfHb are realized indirectly through the binding of endogenous nitric oxide (NO), which leads to endothelial dysfunction, impaired microcirculation, and promotes stimulation of leukocyte adhesion [6]. Arginase released during hemolysis catalyzes the synthesis of ornithine from l-arginine, a substrate for NO production, thereby reducing NO bioavailability [7].

NO can oxidize cfHb to the less toxic methemoglobin, thereby exerting organoprotective effects [8]. In addition, NO has anti-adhesive properties against leukocytes and platelets, which determines its anti-inflammatory potential [9, 10]. Through interactions with proteins such as soluble guanylate cyclase, protein kinase G, and mitochondrial K-ATP channels, NO has been shown to exert a protective effect under conditions of ischemia and reperfusion injury [11]. Given the likely deficiency and reduced bioavailability of NO in patients undergoing CPB, this may provide a rationale for adding this gas to the oxygenator to directly affect the blood and potentially improve clinical outcomes in cardiac surgery.

In recent years, the number of experimental and clinical studies investigating the properties of NO when added to an CPB oxygenator has increased significantly [12, 13]. However, the studies aimed at analyzing the interaction of NO with polypropylene, a polymer of hollow fibers of oxygenator membranes, are insufficient. For example, we are aware of only one study that examined the effect of nitric oxide on the gas exchange and structural integrity of a polypropylene membrane oxygenator. This study showed that NO and its byproduct NO_2 did not affect the structural integrity or gas exchange in a polypropylene membrane oxygenator during 6 hours of CPB *in vitro* [14].

In view of the above, the aim of this study was to investigate the effect of high concentrations of nitric oxide on polypropylene hollow fibers of oxygenators of CPB devices.

Materials and Methods

The study was conducted in two stages. In the first stage, the stability of hollow polypropylene fiber oxygenator membranes in the presence of NO in an air-oxygen mixture was evaluated. For this purpose, their mass transfer characteristics were extensively studied using an experimental unit coupled with a mass spectrometry system. The membrane material was then characterized by infrared spectroscopy. In the second step, an animal experiment was performed using a CPB machine, where an oxygen-air mixture containing NO at a concentration of 100 parts per million (ppm) was supplied.

Comprehensive stability testing of hollow polypropylene fiber membranes in the presence of air-oxygen gas mixture containing NO. The research was carried out in the world-class SMART Laboratory of Polymer Materials and Technologies of D. I. Mendeleev Russian University of Chemical Technology. We developed a unique experimental bench for testing the stability of the polypropylene hollow fiber membrane of the Inspire 8M Sorin oxygenator (LivaNova, Italy) in a gas mixture containing NO (its schematic diagram is shown in Fig. 1).

The experimental bench included a system of gas flow regulators connected to the mixing chamber, a thermostated container of distilled water, a container of 0.9% saline solution, an analytical complex represented by a mass spectrometer with two vacuum stations.

A reciprocating air compressor Remeza SB4/C-24.OLD10 (Remeza, Belarus) with built-in pressure regulator provided compressed air supply through the filtration system (mechanical filter, water separation unit) into the gas flow regulator Bronkhorst El-Flow Prestige (Bronkhorst, Netherlands), then compressed air with known flow rate was supplied to the mixing chamber. Oxygen was also delivered through a gas reducer to a similar gas flow controller, which in turn communicated with the mixing chamber. The gas mixture of N_2 and NO was injected in a similar manner. Thus, the gas mixture to be injected into the membrane contactor was prepared by dynamic flow mixing. The 0.9% NaCl solution was pumped from a glass container to the oxygenation system.

Thus, as a result of simultaneous injection of gas mixture and 0.9% NaCl solution into the oxygenation system at the membrane contactor (hollow fiber membrane of the oxygenator), the contact of two phases was implemented. The components of the gas mixture were partially dissolved in the 0.9% NaCl solution, and the undissolved part of the gas mixture was removed from the membrane contactor unit through a special nipple installed on the body of the oxygenator. The nipple was connected to a

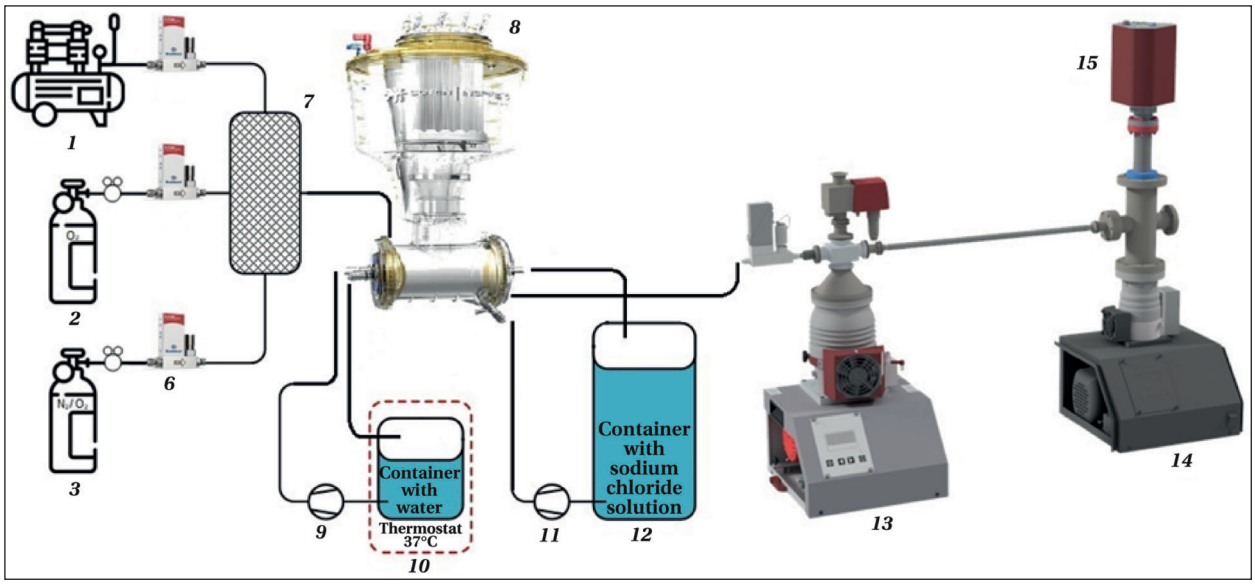

Fig. 1. Schematic diagram of the experimental bench for membrane contactor stability testing.
Note. *1* — air compressor; *2* — cylinder with O_2; *3* — cylinder with mixture of N_2 and NO; *4, 5, 6* — gas flow regulators; *7* — mixing chamber; *8* — oxygenation system; *9, 11* — pumps; *10* — thermostated container with distilled water; *12* — container with 0.9% NaCl solution; *13* — high performance vacuum station; *14* — high vacuum station; *15* — mass spectrometer.

high-precision Bronkhorst El-Flow Metal Sealed gas flow regulator (Bronkhorst, The Netherlands) through which the gas mixture leaving the membrane contactor was injected into the analytical complex. The analytical complex consisted of a Pfeiffer PrismaPro QMG 250 M2, 1-200 u mass spectrometer (Pfeiffer, Germany) coupled with a Pfeiffer HiCube 300 H Eco high vacuum station (Pfeiffer, Germany) for creating a vacuum in front of the mass spectrometer chamber and a Pfeiffer HiCube 80 Eco high vacuum station (Pfeiffer, Germany) for creating a high vacuum directly in the mass spectrometer chamber. The official software developed by the manufacturer Bronkhorst (Netherlands) was used to control the gas flow control system.

First, a simulation of the operating mode was performed, which implied the introduction of a gas mixture of N_2/O_2 = 50/50 vol.% and 0.9% NaCl solution into the oxygenation system for 6 hours. The composition of the gas mixture at the outlet of the membrane contactor of the oxygenation system was measured as a function of the duration of the experiment. Throughout the experiment, the dependence of the composition of the gas mixture at the outlet of the membrane contactor of the oxygenation system on the duration of the experiment was recorded using a mass spectrometric complex. In this way, we determined the composition of the gas mixture in the stationary mode of operation of the oxygenator, which allowed us to determine the separation factor of the system.

Furthermore, the gas mixture $N_2/O_2/NO$ in the ratio of 49.975/49.975/0.05 vol.% and 0.9% NaCl solution was fed into the system, which was preliminarily operated under the mixture N_2/O_2 of 50/50 vol.%. The rest of the experimental conditions were similar to those described above. As in the previous case, the dependence of the concentrations of the gas mixture components on the duration of the experiment was determined throughout the experiment. Since the NO concentration in the feed stream was low (0.05 vol.% (500 ppm)), this value in the gas stream leaving the oxygenator could not be representative. Under these circumstances, the change in gas transport characteristics (separation factor) was evaluated by the change in nitrogen and oxygen concentrations normalized to 100%.

To evaluate the stability of the membrane contactor (hollow fiber membrane of the oxygenator), a parameter characterizing the mass transfer in the system as a whole was used. Such a parameter was the separation factor, which was calculated from the ratio of the concentrations of the components of the mixture in two different gas streams of the system. According to the results of determining the dependence of N_2 and O_2 concentrations in the gas stream leaving the membrane contactor unit of the system, the composition of the gas mixture in the steady state (stationary) mode of operation was determined.

Based on these experimental data, the separation factor was calculated using the following formula:

$$SF = \frac{x_{N_2,r}/x_{O_2,r}}{x_{N_2,f}/x_{O_2,f}}$$

where $x_{N2,r}$ — nitrogen concentration in the retentate flow vol.%; $x_{O2,r}$ — oxygen concentration in

retentate flow, vol.%; $x_{N2,f}$ — nitrogen concentration in the feed stream, vol.%; $x_{O2,f}$ — oxygen concentration in the feed stream, vol.%. In this case, the gas stream leaving the membrane unit of the oxygenation system was the retentate stream. Thus, to determine the separation factor of the system, the ratio of N_2 to O_2 concentration in the retentate stream and feed stream was calculated.

Fourier transform infrared spectra in the range of 4000–700 cm^{-1} were recorded for additional testing of the membranes and to investigate the possible chemical interactions with NO. The analysis was performed on an IRAffinity-1 instrument (Shimadzu, Kyoto, Japan) at ambient temperature with a resolution of 4 cm^{-1} using an HATR accessory (Pike, USA). An Inspire 8M Sorin polypropylene hollow fiber oxygenator membrane (LivaNova, Italy) was used in this study. Twenty scans were signal-averaged to obtain the results.

Experimental animal study using NO-containing air-oxygen mixture delivered to the oxygenator of the heart-lung machine. This study was approved by the Bioethics Committee of the Almazov National Medical Research Center (protocol PZ_22_6_V2 dated 08.06.2022) and was conducted at the Center for Preclinical Translational Research of the Almazov National Medical Research Center.

Animals. Ten female domestic landrace pigs, aged 3 to 4.3 months, were included in the study. The mean body weight was 38.9 (37.7; 40.9) kg. The animals were divided into two groups, control and experimental. In the experimental group, 100 ppm NO was added to the air-oxygen mixture in the oxygenator during CPB. No NO was added to the oxygenator in control group animals. All animals underwent CPB for 4 hours and were observed for 12 hours. All animals were then removed from the experiment.

Anesthesia and perfusion support. Combined anesthesia was performed using general combined anesthesia and regional anesthesia (intercostal nerve block). Premedication included an intramuscular injection of zolazepam/tiletamine (Zoletil Virbac, France) 20 mg/kg. Under aseptic conditions, the peripheral vein (auricular vein) was punctured and catheterized with an 18–20 G catheter. After induction of anesthesia with propofol (Propofol-Lipuro, B. Braun, Germany) 2–3 mg/kg, direct laryngoscopy and tracheal intubation were performed. After tracheal intubation, the non-depolarizing myorelaxant rocuronium bromide was administered at a dose of 0.6–1.2 mg/kg. Anesthesia was maintained by inhalation of 1.5–2.5 vol.% isoflurane (Aerran Baxter Healthcare Corporation, USA) using a Heyer Medical AG vaporizer (Dräger, Germany). Jugular vein catheterization was performed under ultrasound guidance in all animals to ensure central venous pressure (CVP) monitoring and drug infusion. Invasive blood pressure (BP) monitoring was performed by femoral artery catheterization with a 20G B. Braun catheter (20G B. Braun) using the Seldinger technique. A 10-F Nelaton urinary catheter was inserted to control the rate and pattern of urine flow.

Vital signs were monitored using the Mindray BeneView T8 monitoring system (Mindray, PRC). Monitoring during the experiment included pulse oximetry, electrocardiography (ECG), measurement of central temperature, invasive BP and CVP, gas composition of the inhaled and exhaled mixture, and respiratory rate (RR).

Mechanical lung ventilation (MLV) was performed in normal and normocapnic modes. Mindray Wato Ex-35 anesthesia-breathing circuit (Mindray, PRC) was used for ventilation. The following parameters of intraoperative ventilatory support were used: volume-controlled ventilation (VCV) mode, tidal volume (Vt) 20–30 ml/kg/min, RR 8–14 per minute, and fraction of inspired oxygen (FiO_2) 65%. Ventilation parameters were adjusted based on the results of oximetry, capnometry and arterial blood gas analysis.

Before the main phase of the operation, the regional component of combined anesthesia was administered using nerve block with ropivacaine (Ropivacaine, Pharmzashchita, Russia) at a dose of 5 mg/kg.

Cardiopulmonary bypass during the experiment was provided by a WEL-1000B Plus device (Tianjin Welcome Medical Equipment, PRC) using an Inspire 8M Sorin oxygenator (LivaNova, Italy). Perfusion safety was ensured by monitoring the pressure in the blood lines, the blood level sensor in the cardiotomy reservoir, and the gas bubble sensor. The mandatory components of the primary filling volume (prime) of the extracorporeal circuit were gelofusin (Gelofusin, B. Braun, Germany), heparin (Heparin sodium Braun, B. Braun, Germany) at a dose of 3 U/mL prime and sodium bicarbonate for pH normalization at a dose of 3 mmol/100 mL prime. Heparin was injected at a dose of 300 U/kg prior to CPB. Activated coagulation time (ACT) was measured 5 minutes after heparin administration and CPB was started when target values were reached (ACT>480 sec). The volume perfusion rate was 3 L/min/m^2. The initial gas flow was 2 L/min and was adjusted based on blood gas analysis. Blood gas control was performed in α-stat mode. To maintain hypocoagulation, heparin was administered as needed at a dose of 100–200 units/kg, and ACT was measured every 30 minutes. Adequacy of CPB was assessed by mean blood pressure (50–80 mm Hg), blood gas analysis, and acid-base balance. Normal temperature was maintained with a heat exchanger connected to the oxygenator with a target temperature of 37.5–38°C. Heparin reversal with protamine sulfate was avoided in favor of thorough surgical hemostasis. If protamine sulfate (Protamine Sulfate, Ellara, Russia) was used, its dose was cal-

culated on the basis of a ratio of 1–1.3 mg of protamine sulfate per 100 units of heparin initially administered. The calculated dose of protamine sulfate was administered over 20 minutes.

In the postoperative period, all animals received prolonged inhalational anesthesia with isoflurane and stable hemodynamic parameters were maintained. Protective ventilation, inotropic and vasopressor support were provided as indicated. Vital signs monitoring was continued.

Surgical procedure. A left-sided thoracotomy was performed in the 3rd intercostal space. After reaching the target level of hypocoagulation, an aortic cannula was successively inserted into the ascending aorta (20 Fr aortic cannula, Medtronic, USA) and a venous cannula into the right atrial cavity through the auricle (31 Fr venous cannula, Medtronic, USA). After weaning from CPB and removal of the cannula, hemostasis was checked. After drainage into the left pleural cavity with entry into the pericardial cavity, the wound was sutured layer by layer.

NO supply to the CPB circuit. In the experimental group of animals, during the whole period of CPB, synthesized NO from the experimental sample of the plasma-chemical synthesis unit was fed into the main line of air-oxygen mixture delivery to the oxygenator at 100 ppm [15, 16]. Animals of the control group were supplied with air-oxygen mixture without NO through the CPB oxygenator. The air-oxygen mixture was delivered to the «gas in» port of the oxygenator through ¼ diameter PVC tubing. Three-way stopcocks Discofix C (B. Braun, Germany) were previously installed in the supply line: a NO supply tube was connected 10 cm before the oxygenator and a tube for monitoring NO and NO_2 concentrations was connected 5 cm after the oxygenator (Fig. 2).

To control the gas composition of the mixture supplied to the oxygenator, NO and NO_2 were monitored both before and after the oxygenator. The upper limit for NO_2 in the oxygenator circuit was set at 2 ppm. If this value was exceeded, the supply of NO was stopped. The levels of NO and NO_2 in the gas mixture supply line to the oxygenator were continuously monitored. NO and NO_2 levels at the oxygenator outlet were monitored discreetly at 30-minute intervals.

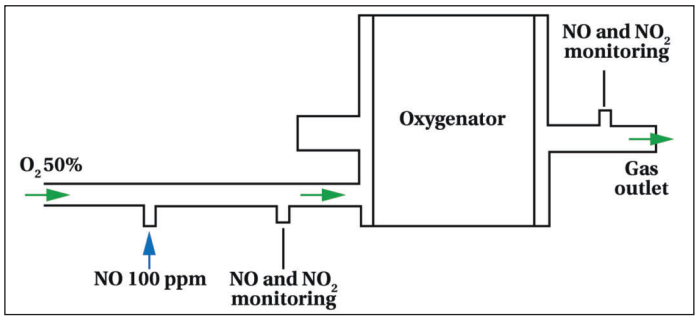

Fig. 2. Scheme of supply of air-oxygen mixture and NO to the oxygenator and monitoring of NO, NO_2.

Methemoglobin concentration determination. To assess the safety of high-dose NO delivery to the oxygenator, methemoglobin levels were analyzed. Its changes were studied at three time points: 2 hours after the start of CPB, at weaning from CPB, 6 hours after weaning.

Testing for leakage of the samples of membrane contactors of oxygenators. Samples of Inspire 8M polypropylene oxygenators (LivaNova, Italy) were leak tested to detect internal damage to the oxygenator. Simultaneous injection of compressed air and 0.9% NaCl solution into the oxygenation system in the membrane contactor simulated the working process. As part of the experiment, the gas pressure was gradually increased from 0.1 to 0.5 megapascals (MPa) in increments of 0.1 MPa. Thus, two phases such as pressurized gas and 0.9% NaCl solution contacted through the porous membrane. The gas dissolved in the 0.9% NaCl solution was desorbed in the container from which the solution was injected. 3 samples of Inspire 8M oxygenators previously used in animal studies (2 from the main group, 1 from the control group) were tested for leakage.

Scanning electron microscopy of the surface of hollow fiber samples of membrane contactors. The surface of hollow fiber samples of polypropylene oxygenator membranes used during CPB in experimental animals was analyzed by scanning electron microscopy (SEM). Three Inspire 8M oxygenator membrane samples (2 from the main group, 1 from the control group) were examined. After weaning from CPB, the oxygenators were washed with 10 liters of 0.9% NaCl solution and then filled with 2% glutaric aldehyde for fixation [13]. A JEOL 1610LV scanning electron microscope (JEOL, Japan) was used. As a result, a series of microphotographs of the hollow fiber surface were obtained at different magnifications ranging from ×45 to ×15,000.

Statistical analysis was performed using the MedCalc Statistical Software 20.218 package (MedCalc Software Ltd, Belgium). Because of the small sample size, nonparametric methods were used. The Mann–Whitney *U* criterion for independent groups and the Wilcoxon test for dependent groups were used to compare numerical parameters. Multigroup comparisons were performed using the Bonferroni correction. Data were presented as median *(Q1; Q3)*. A two-sided *P* level of significance was used. The critical level of significance was set at *P*=0.05.

Results

Extensive testing of the stability of polypropylene oxygenator membranes in the presence of an air-oxygen mixture containing NO.

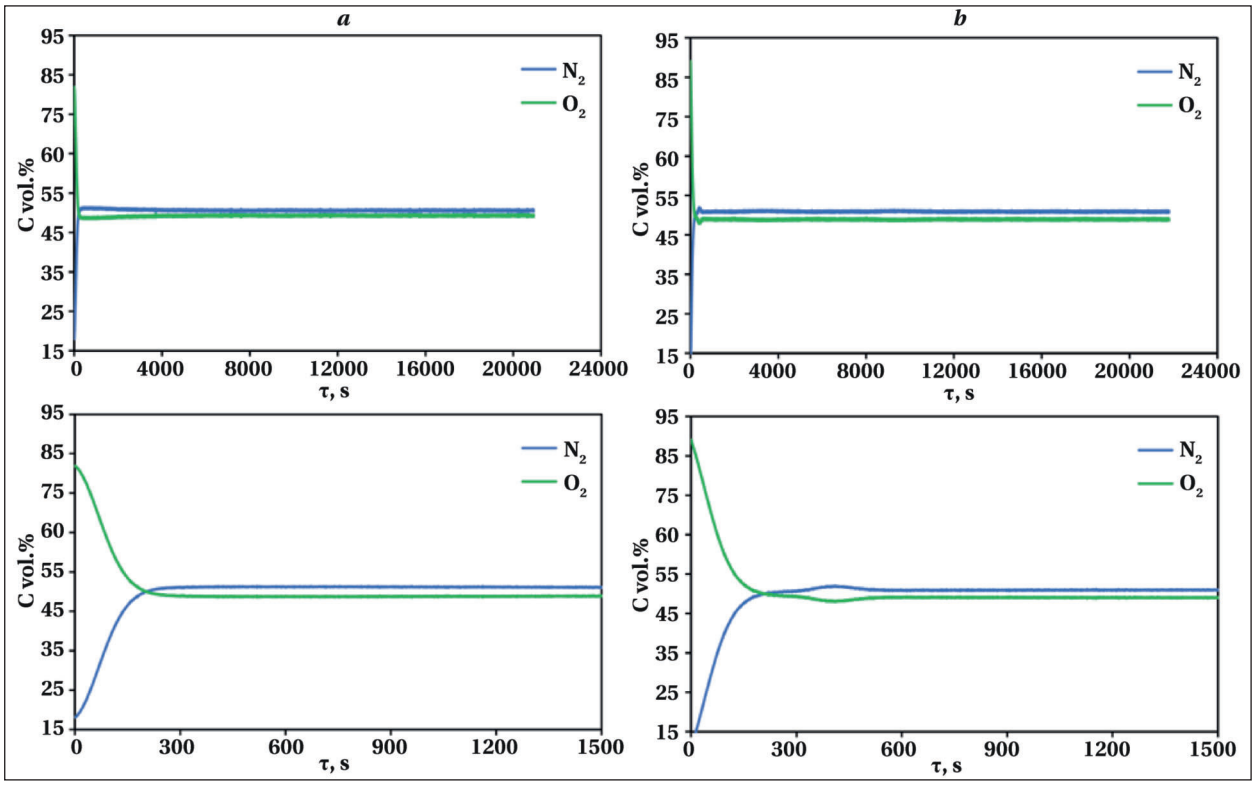

Fig. 3. Relationship between the concentration (vol.%) of N_2 and O_2 in the outlet flow of the Inspire 8M membrane unit and the time of the experiment.

Note. Simulation of the working process using 0.9% NaCl water solution with passing of gas mixture of *(a)* N_2/O_2 50/50 vol.%; *(b)* N_2/O_2/NO 49.975/49.975/0.05 vol.%.

Determination of gas transport characteristics of polypropylene oxygenator. According to the results of the analysis of the composition of the gas stream leaving the membrane unit of the oxygenator, the concentrations of N_2 and O_2 in the steady state of operation of the oxygenator were determined. The graphs of the changes in the concentrations of N_2 and O_2 in the gas stream leaving the membrane unit of the oxygenation system, depending on the duration of the experiment, are shown in Fig. 3.3. The results showed that the composition of the gas mixture leaving the membrane unit of the oxygenation system had a slight difference from the raw stream, i. e., the concentration of N_2 increased by 0.83 vol.%. When comparing the composition of gas mixtures at the outlet of the oxygenator for N_2/O_2 and N_2/O_2/NO mixtures, a minor increase in N_2 concentration (by 0.38 vol.%) was found. In addition to the graphs of the dependence of the composition of the gas mixture on the duration of the experiment, Fig. 4 shows the mass spectra of the gas mixture at the outlet of the membrane unit of the oxygenator. During the experiment with the gas mixture containing NO, a characteristic peak at m/z = 30 was detected in the mass spectra, confirming the presence of this component in the system. The averaged values of N_2 and O_2 concentrations in the gas stream leaving the membrane unit of the oxygenation system (for steady-state operation mode) and the calculated values of the separation factor are presented in Table 1.

Thus, based on the results obtained, it can be concluded that the Inspire 8M oxygenator maintains its mass transfer characteristics in the presence of 500 ppm NO in the operating gas mixture for at least 6 hours.

Analysis of IR spectra (Fourier Transform) obtained from the study. In order to determine possible

Table 1. Concentrations of N_2 and O_2 at the outlet from the membrane unit of the oxygenation system and the separation factor.

Model of oxygenation system	$x_{N_2, r}$, vol.%	$x_{O_2, r}$, vol.%	SF
N_2/O_2 gas mixture			
Inspire 8M	50.83	49.17	1.03
N_2/O_2/NO gas mixture			
Inspire 8M	51.21	48.79	1.05

Note. $x_{N_2, r}$ — concentration of nitrogen in the retentate flow, vol.%; $x_{O_2, r}$ — concentration of oxygen in the retentate flow; SF — separation factor.

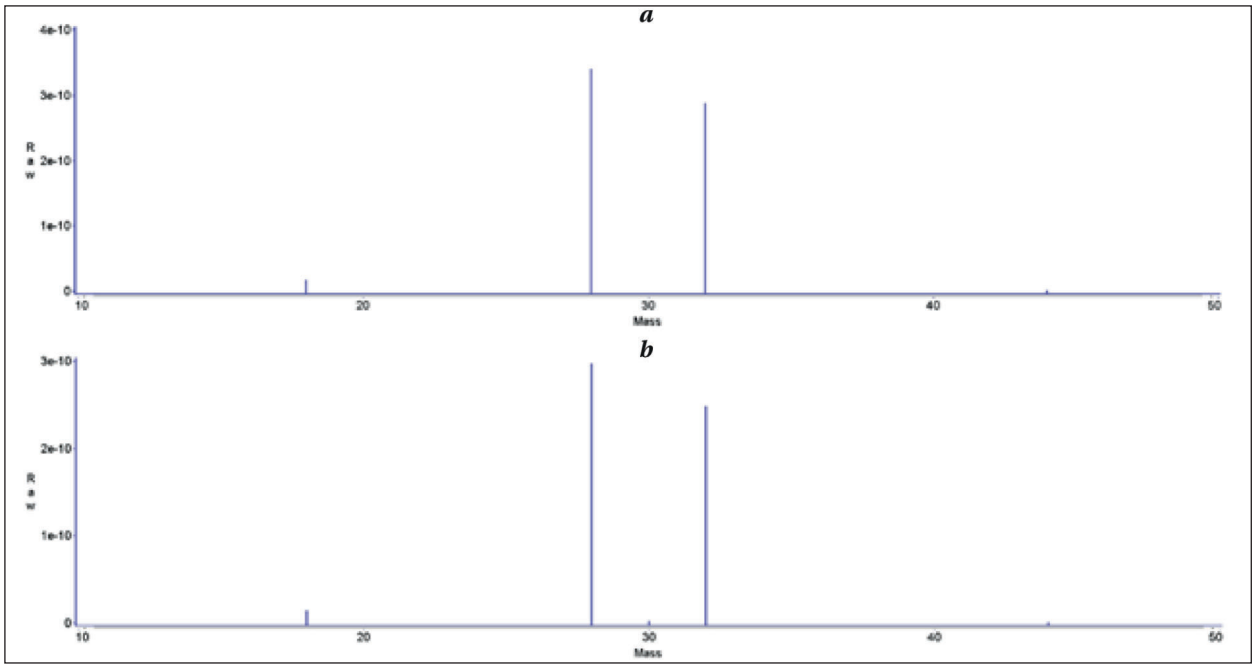

Fig. 4. Mass spectrum of the outlet flow of the Inspire 8M membrane unit.
Note. Simulation of the working process using 0.9 % NaCl water solution with passing of gas mixture of *(a)* N_2/O_2 50/50 vol. %; *(b)* N_2/O_2/NO 49.975/49.975/0.05 vol. %.

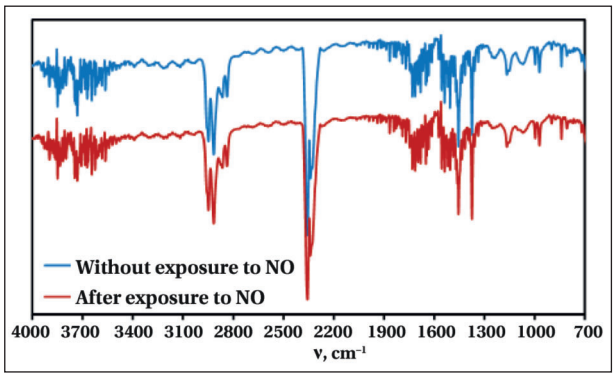

Fig. 5. IR spectra (Fourier transform) of hollow polypropylene membranes before and after exposure to a gas mixture containing NO.

chemical reactions of NO and polypropylene of the oxygenator hollow membranes, the results of IR spectroscopy were subjected to Fourier transformation (Fig. 5). The data obtained indicated that the membrane material did not undergo any changes when exposed to NO, which was confirmed by the absence of characteristic band shifts in the IR spectrum of the polypropylene of the hollow membrane reflecting NO chemisorption. There was also no evidence of physical adsorption of NO on polypropylene. According to the library of the National Institute of Standards and Technology (NIST) (USA), the peak of the IR spectrum «responsible» for bond vibrations in the NO molecule is in the range of 1830–1900 cm^{-1}. No differences were found between control samples of polypropylene membrane and samples exposed to 500 ppm NO. Thus, it can be concluded that the presence of 500 ppm NO in the air-oxygen mixture has no effect on polypropylene hollow fibers.

Results of an experimental animal study with NO supply to the oxygenators of the heart-lung machine. *Analysis of NO and NO_2 concentrations at the inlet and outlet of the oxygenator.* According to the study protocol, 5 cardiac surgeries were performed on pigs under cardiopulmonary bypass with NO supplied to the polypropylene oxygenator circuit. 100 ppm NO was delivered to the Inspire 8M oxygenator during 240 minutes of CPB. By operating the experimental device in automatic mode with a

Table 2. NO concentration at the oxygenator inlet and outlet during CPB, median *(Q1; Q3)*, *N*=5

Time from the beginning of CPB, min	NO_2 at the oxygenator inlet, ppm	NO_2 at the oxygenator outlet, ppm	*P* Mann–Whitney *U* test
10	99.5 (98.5; 103.3)	56.1 (35.3; 61.6)	*P*=0.008
40	99.4 (98.1; 100.1)	56.8 (52.3; 60.2)	*P*=0.008
70	99.8 (98; 100.6)	59 (54.8; 61.3)	*P*=0.008
100	99.1 (97.9; 100.2)	61.8 (54.2; 62.7)	*P*=0.008
130	100 (91.2; 100.3)	63.9 (53.2; 67.5)	*P*=0.032
160	99.7 (99.2; 101.4)	63.2 (61.4; 67.2)	*P*=0.008
190	100.1 (99.8; 100.5)	65.4 (63.7; 67.3)	*P*=0.029
220	99.7 (99.4; 100.4)	65.2 (64.5; 67.4)	*P*=0.008
240	100.4 (100.2; 101.2)	68.9 (67.8; 72.1)	*P*=0.009

Table 3. NO_2 concentration at the inlet and outlet of the CPB oxygenator during CPB. median *(Q1; Q3)*, *N*=5

Time from the beginning of CPB, min	NO_2 at the oxygenator inlet, ppm	NO_2 at the oxygenator outlet, ppm	*P* Mann–Whitney *U* test
10	1.45 (0.9; 1.85)	1.15 (0.7; 1.2)	*P*=0.237
40	1.75 (1.4; 1.9)	1.1 (1.1; 1.15)	*P*=0.024
70	1.7 (1.4; 1.85)	1.2 (1.1; 1.6)	*P*=0.191
100	1.1 (1.08; 1.25)	1.5 (0.7; 1.2)	*P*=0.8
130	1.8 (1.65; 1.8)	1.3 (1; 1.6)	*P*=0.045
160	1.5 (1.4; 1.65)	1.2 (1; 1.35)	*P*=0.06
190	1.65 (1.5; 1.75)	1.25 (1.1; 1.35)	*P*=0.029
220	1.45 (1.35; 1.7)	1.3 (1.15; 1.5)	*P*=0.026
240	1.6 (1.45; 1.65)	1.4 (1.38; 1.6)	*P*=0.366

target NO level of 100 ppm at the oxygenator inlet, an average NO concentration of 99.2±5.6 ppm was maintained, ranging from 95.7 to 111.3 ppm. We found significant differences in NO levels at the oxygenator inlet and outlet at all stages of measurement during CPB (Table 2). In the total sample of 45 measurements, the mean NO concentration at the outlet was 60.5±9.6 ppm, which was significantly lower than the concentration at the oxygenator inlet ($P<0.0001$). The median reduction in outlet NO concentration was 36.7 (33.7; 40.7) ppm.

There was a tendency for increased NO levels at the outlet at the time of weaning from CPB, but these differences were not statistically significant. The patterns found suggest a possible significant absorption of NO by the blood during delivery to the oxygenator, reaching 35–50%.

Data on NO_2 levels in the gas mixture supply line to the polypropylene oxygenator and in the outlet are shown in Table 3. Analyzing these parameters, we found stable values of NO_2 level during CPB, without any tendency to increase. The lower level of NO_2 in the outlet, which was statistically significant at several stages of the study, was also revealed.

In a total sample of 45 measurements of NO_2 concentration in the gas mixture supply line to the oxygenator, the mean value of the parameter was 1.58±0.28 ppm, which was significantly higher than the values obtained in the oxygenator outlet, 1.22±0.26 ppm ($P<0.001$). The median decrease in NO_2 concentration at the outlet was 0.4 (0.2; 0.7) ppm.

Four instances of NO_2 exceeding 2 ppm were noted during the study. In these situations, NO delivery was stopped and resumed after NO_2 was reduced to less than 1 ppm. There were no other instances of NO discontinuation.

Analysis of methemoglobin changes. Data on methemoglobin levels during CPB are shown in Table 4. As can be seen from the above data, 100 pm NO delivery to the polypropylene oxygenator was not associated with a significant increase in methemoglobin levels. Methemoglobin levels did not exceed the upper limit of acceptable values (3%), and no significant differences were found compared to the control group.

Table 4. Changes in methemoglobin percentage (%) during CPB-assisted cardiac surgery, median *(Q1; Q3)*, *N*=10

Stage of the study	Methemoglobin, %	
	Control group, *N*=5	NO group, *N*=5
Baseline	0.9 (0.7; 1.1)	0.9 (0.8; 1.1)
2 hours of CPB	0.8 (0.4; 0.9)	1.1 (1; 1.4)
4 hours of CPB (end of CPB)	1.9 (1.2; 1.9)	1.5 (1.1; 2.2)

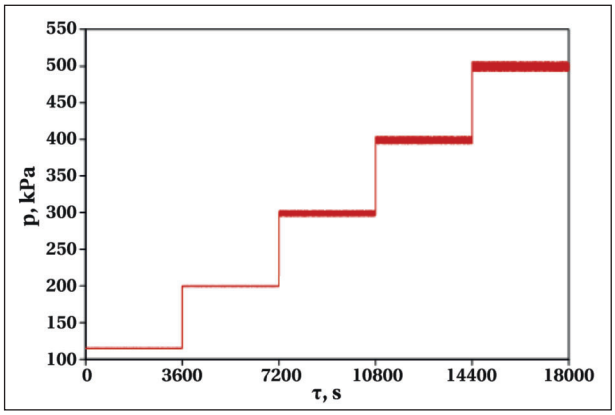

Fig. 6. Graph of the gas pressure at the outlet of the membrane contactor of the oxygenation system as a function of the duration of the experiment with incremental pressure increase in the system.

Leakage testing of oxygenator membrane contactor samples. Three Inspire 8M oxygenators previously used during animal CPB (2 from the main group, 1 from the control group) were tested. Fig. 6 shows the relationship between the pressure inside the gas-filled membrane contactor and the duration of the experiment. During this experiment, no significant differences were found in the functioning of the three samples studied and no significant variation in the gas pressure at the outlet of these samples was detected. No episodes of pressure drop were observed at any stage of the study. The data are presented as averaged gas pressure values at the outlet of the membrane contactor for the three samples studied.

Scanning electron microscopy of the surface of polypropylene hollow fiber membrane samples. Micrographs of polypropylene hollow fiber membrane samples after procedures performed on experimental animals are shown in Fig. 7.

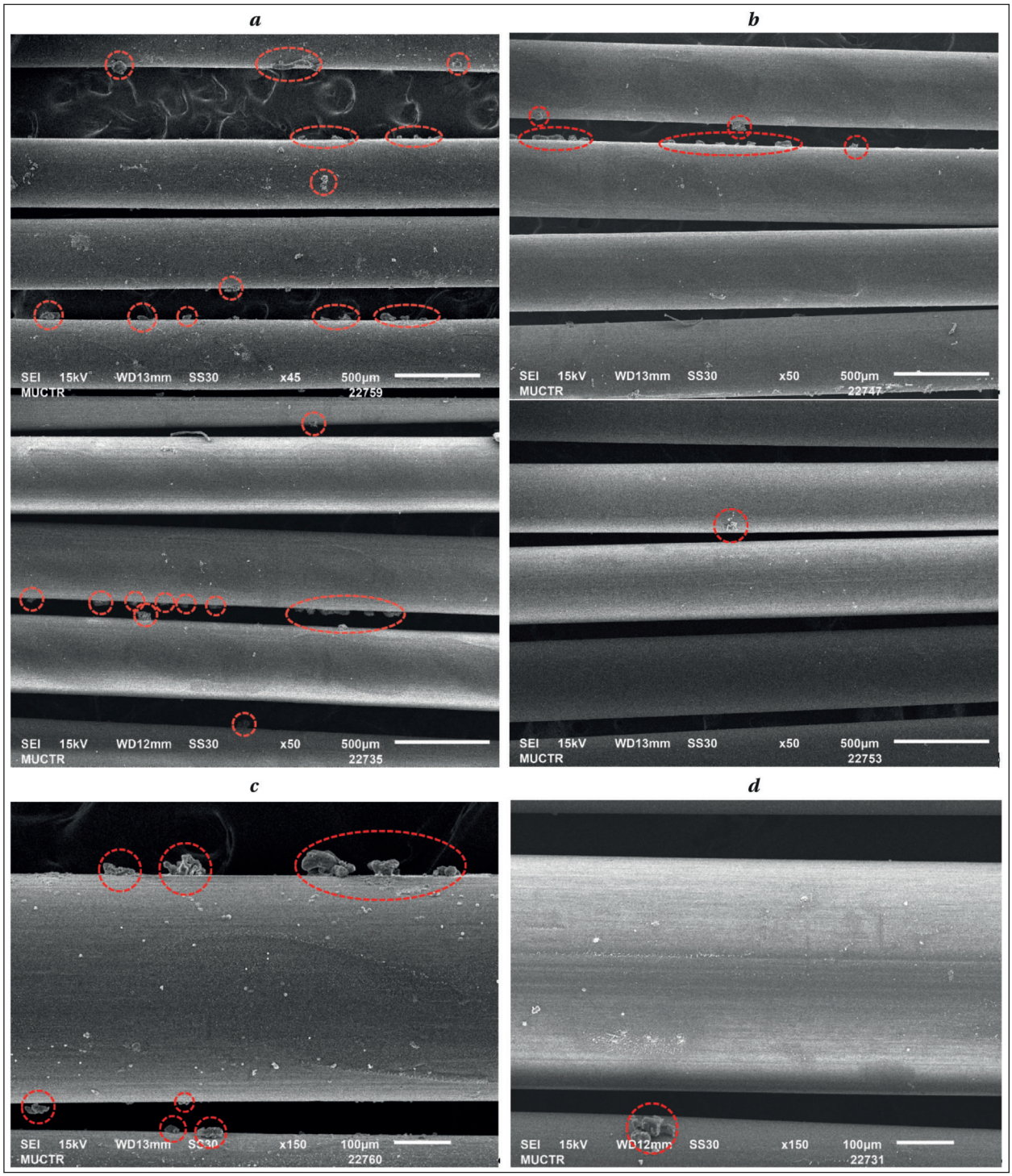

Fig. 7. Scanning electron micrographs of the surface of polypropylene hollow fiber membranes obtained by scanning electron microscopy.
Note. *a* — control group; *b* — NO supply group; *c* — control group; *d* — NO group. Magnification: *a* — ×45 and ×50; *b* — ×50; *c*, *d* — ×150. Fibrin deposits are circled in red.

The comparison of micrographs of the surface of hollow fibers of polypropylene membranes in control samples (without NO exposure) and samples after CPB with NO supply to the oxygenator showed that the surface of membranes of control samples had a significantly higher number of clots resulting from exposure to blood. For example, micrographs of the membrane surface of the control sample (Fig. 7, *a*) showed 12 (micrograph with ×45 approximation) and 10 (micrograph with ×50 approximation) groups of fibrin deposits. Micrographs of membrane samples exposed to nitric oxide showed fewer fibrin deposits (Fig. 7, *b*). The first ×50 magnification image demonstrated 5 groups of such deposits, while the second image showed 1 group.

The ×150 magnification micrographs of the samples support the above statement. On the surface of the control group specimen (Fig. 7, *c*), 7 groups of fibrin deposits were present, while only 1 group of deposits was found on the surface of the fiber of the nitric oxide-exposed specimen (Fig. 7, *d*).

Thus, examination of the surface of hollow fibers of polypropylene membranes by SEM revealed fewer fibrin clots when 100 ppm NO was supplied to the oxygenator compared to control samples.

Discussion

The polypropylene hollow fiber oxygenator was shown to retain its gas transport characteristics after six hours of exposure to an air-oxygen mixture containing 500 ppm NO. The composition of the gas mixture at the outlet of the membrane unit of the oxygenator was not significantly different from the composition of the supplied mixture. The results of the study conducted indicate that exposure to NO at such a high concentration does not affect mass transfer in the polypropylene hollow fiber oxygenator. In addition, FTIR spectroscopy was used to demonstrate for the first time that 500 ppm NO does not affect the structure of polypropylene membranes.

These findings support the results of the only study to investigate the effects of NO on polypropylene oxygenators. S.C. Body et al. investigated the effects of NO on gas exchange and structural integrity of a polypropylene membrane oxygenator. Nine membrane oxygenators were exposed to 224±10 ppm NO and 6.7±1.7 ppm NO_2 and 73% O_2 in nitrogen, and six oxygenators were exposed to 73% O_2 in nitrogen. Heparinized thrombocytopenic bovine blood was circulated in a closed circuit for 6 hours.

Comparison of O_2 or CO_2 transfer rates between groups at 0, 1, 3, and 6 hours showed no differences. No oxygenators «failed» hydraulic integrity tests or malfunctioned during the experiment. There was no evidence of degradation of the experimental material in the appearance of the oxygenators. There were also no differences in blood parameters. Pressure gradients in the oxygenators did not differ between groups at any time point and did not change over time. SEM revealed no differences in pore size, membrane structure, or loss of structural integrity, even in oxygenators subjected to further mechanical damage during structural integrity testing. However, it should be noted that potential chemical interactions between polypropylene and NO were not investigated in the above work [14].

In the second stage of our study, cardiac surgery was performed on animals under CPB with NO supplied to the circuit of the polypropylene oxygenator. We found significant differences in NO concentration at the inlet of the oxygenator and in the outlet circuit at all stages of CPB, confirming the saturation of animal blood with NO. In addition, an increase in NO concentration at the oxygenator outlet was observed during CPB, with a median of 56.1 (35.3; 61.6) at the 10^{th} minute of CPB and a median of 68.9 (67.8; 72.1) at the 240^{th} minute of CPB. The data obtained suggest that at a certain stage of CPB, the NO deposition mechanisms are exhausted, making further saturation impossible.

A review of the literature showed that most clinical studies investigating the effects of NO added to the oxygenator of a CPB machine typically use NO concentrations of 20–40 ppm [17–20]. We showed that the addition of 100 ppm NO during CPB was not associated with an increase in NO_2 concentration to the toxic level of 2 ppm in 91% of the measurements with a mean value of 1.58±0.28 ppm. In the aforementioned study, J. Body et al. showed that NO supply at a concentration of 224±10 ppm did not damage the membrane of a polypropylene oxygenator, but was associated with an increase in NO_2 concentration up to 6.7±1.7 ppm [14], which is a toxic concentration for living organisms. Therefore, it can be assumed that the NO concentration of 100 ppm may be safe for use under clinical conditions.

Analysis of methemoglobin concentrations did not reveal values exceeding the acceptable limits throughout the study. Intergroup comparisons of methemoglobin levels showed no significant differences or trends, which is not consistent with the pharmacokinetic concepts of NO therapy or the results of previous studies. For example, in the first clinical study on the effects of 100 ppm NO delivered to the oxygenator, including 47 patients, the methemoglobin concentration was significantly higher in the main group compared to the control group [21]. Our results may be due to the different susceptibility of human and animal blood to hemolysis.

All investigated oxygenators were tested for leakage and no damage to the integrity of the membrane material and the device body was found. Scanning electron microscopy of hollow polypropylene fiber oxygenators was performed for the first time after NO delivery in an in vivo experiment.

The SEM results after 4 hours of CPB demonstrated the beneficial effects of NO addition to the oxygenator. Oxygenators in the control group were characterized by more fibrin deposition than oxygenators in the main group.

Conclusion

This comprehensive study has found no negative effect of 500 ppm NO on the membranes of polypropylene hollow fiber oxygenators. In an animal study with 100 ppm NO supplied to the oxygenator, no levels of NO_2 or methemoglobin were found to exceed acceptable safe levels. A decrease in the intensity of fibrin deposition on the hollow fibers of polypropylene oxygenator membranes was observed after NO addition.

References

1. *Patel V., Unai S., Gaudino M., Bakaeen F.* Current readings on outcomes after off-pump coronary artery bypass grafting. *Semin Thorac Cardiovasc Surg.* 2019; 31 (4): 726–733. DOI: 10.1053/j.semtcvs.2019.05.012. PMID: 31125606.
2. *Bronicki R. A., Hall M.* Cardiopulmonary bypass-induced inflammatory response: pathophysiology and treatment. *Pediatr Crit Care Med.* 2016; 17 (8 Suppl 1): S272–278. DOI: 10.1097/PCC.0000000000000759. PMID: 27490610.
3. *Wetz A. J., Richardt E. M., Schotola H., Bauer M., Bräuer A.* Haptoglobin and free haemoglobin during cardiac surgery-is there a link to acute kidney injury? *Anaesth Intensive Care.* 2017; 45 (1): 58–66. DOI: 10.1177/0310057X1704500109. PMID: 28072936.
4. *Datt V., Wadhhwa R., Sharma V., Virmani S., Minhas H. S., Malik S.* Vasoplegic syndrome after cardiovascular surgery: a review of pathophysiology and outcome-oriented therapeutic management. *J Card Surg.* 2021; 36 (10): 3749–3760. DOI: 10.1111/jocs.15805. PMID: 34251716.
5. *Di Masi A., De Simone G., Ciaccio C., D'Orso S., Coletta M., Ascenzi P.* Haptoglobin: from hemoglobin scavenging to human health. *Mol Aspects Med.* 2020; 73: 100851. DOI: 10.1016/j.mam.2020.100851. PMID: 32660714.
6. *Schaer C. A., Deuel J. W., Schildknecht D., Mahmoudi L., Garcia-Rubio I., Owczarek C., Schauer S., et al.* Haptoglobin preserves vascular nitric oxide signaling during hemolysis. *Am J Respir Crit Care Med.* 2016; 193 (10): 1111–1122. DOI: 10.1164/rccm.201510-2058OC. PMID: 26694989.
7. *Steppan J., Tran H. T., Bead V. R., Oh Y. J., Sikka G., Bivalacqua T. J., Burnett A. L., et al.* Arginase inhibition reverses endothelial dysfunction, pulmonary hypertension, and vascular stiffness in transgenic sickle cell mice. *Anesth Analg.* 2016; 123 (3): 652–658. DOI: 10.1213/ANE.0000000000001378. PMID: 27537757.
8. *Spina S., Lei C., Pinciroli R., Berra L.* Hemolysis and kidney injury in cardiac surgery: the protective role of nitric oxide therapy. *Semin Nephrol.* 2019; 39 (5): 484–495. DOI: 10.1016/j.semnephrol.2019.06.008. PMID: 31514912.
9. *Galkina S. I., Golenkina E. A., Viryasova G. M., Romanova Y. M., Sud'ina G. F.* Nitric oxide in life and death of neutrophils. *Curr Med Chem.* 2019; 26 (31): 5764–5780. DOI: 10.2174/0929867326266618121309152. PMID: 30543162.
10. *Gresele P., Momi S., Guglielmini G.* Nitric oxide-enhancing or releasing agents as antithrombotic drugs. *Biochem Pharmacol.* 2019; 166: 300–312. DOI: 10.1016/j.bcp.2019.05.030. PMID: 31173724.
11. *Zhang Y. Q., Ding N., Zeng Y.-F., Xiang Y.-Y., Yang M.-W., Hong F.-F., Yang S.-L.* New progress in roles of nitric oxide during hepatic ischemia reperfusion injury. *World J Gastroenterol.* 2017; 23 (14): 2505–2510. DOI: 10.3748/wjg.v23.i14.2505. PMID: 28465634.
12. *Loughlin . M., Browne L, Hinchion J.* The impact of exogenous nitric oxide during cardiopulmonary bypass for cardiac surgery. *Perfusion.* 2022; 37 (7): 656–667. DOI: 10.1177/02676591211014821. PMID: 33983090.
13. *Пичугин В. В., Баутин А. Е., Домнин С. Е., Рязанов М. В., Сандалкин Е. В.* Доставка газообразного оксида азота в экстракорпоральный контур циркуляции: экспериментальные и клинические данные: обзор литературы. *Вестник интенсивной терапии им. А. И. Салтанова.* 2021; 3: 108–116. [*Pichugin V. V., Bautin A. E., Domnin S. E., Ryazanov M. V., Sandalkin E. V.* Delivery of gaseous nitric oxide to the extracorporeal circulation circuit: experimental and clinical data*:* a review. *Ann Crit Care /Vestnik Intensivnoy Terapii im A. I. Saltanova.* 2021; 3: 108–116. (in Russ.)]. DOI: 10.21320/1818-474X-2021-3-108-116.
14. *Body S. C., FitzGerald D., Voorhees C., Hansen E., Crowley C., Voorhees M. E., Shernan S. K.* Effect of nitric oxide upon gas transfer and structural integrity of a polypropylene membrane oxygenator. *ASAIO J.* 1999; 45 (6): 550–554. DOI: 10.1097/00002480-199911000-00008. PMID: 10593685.
15. *Баутин А. Е., Селемир В. Д., Нургалиева А. И., Морозов К. А., Никифоров В. Г., Бикташева Л. З., Афанасьева К. Ю., с соавт.* Ингаляционная терапия оксидом азота, полученным методом синтеза из атмосферного воздуха, в послеоперационном периоде кардиохирургических вмешательств у детей: одноцентровое ретроспективное когортное исследование. *Вестник интенсивной терапии им. А. И. Салтанова.* 2021; 3: 98–107. [*Bautin A. E., Selemir V. D., Nurgalieva A. I., Morozov K. A., Nikiforov V. G., Biktasheva L. Z., Afanasyeva K. Yu., et al.* Inhalation therapy with nitric oxide synthesized from atmospheric air in the postoperative period of cardiac surgery in children: single-center retrospective cohort study. *Ann Crit Care /Vestnik Intensivnoy Terapii im AI Saltanova.* 2021; 3: 98–107. (in Russ.)]. DOI: 10.21320/18-474 X-2021–3–98–107.
16. *Мазурок В. А., Нургалиева А. И., Баутин А. Е., Ржеутская Р. Е., Мазурок А. В., Оразмагомедова И. В., Груздова Д. Г., с соавт.* Объемно-компрессионная осциллометрия для оценки гемодинамики у взрослых с некорригированными врожденными пороками сердца и легочной артериальной гипертензией. *Анестезиология и реаниматология.* 2022; 6: 58–67. [*Mazurok V. A., Nurgalieva A. I., Bautin A. E., Rzheutskaya R. E., Mazurok A. V., Orazmagomedova I. V., Gruzdova D. G., et al.* Volu-

metric compression oscillometry for the assessment of hemodynamics in adults with corrected congenital heart defects and pulmonary arterial hypertension. *Anesthesiol.Reanimatol/ Anesteziologiya i Reanimatologiya*. 2022; 6: 58–67. (in Russ.)]. DOI: 10.17116/ anaesthesiology202206158.

17. *James C., Millar J., Horton S., Brizard C., Molesworth C., Butt W.* Nitric oxide administration during paediatric cardiopulmonary bypass: a randomised controlled trial. *Intensive Care Med*. 2016; 42 (11): 1744–1752. DOI: 10.1007/s00134-016-4420-6. PMID: 27686343.
18. *Kamenshchikov N. O., Anfinogenova Y. J., Kozlov B. N., Svirko Y. S., Pekarskiy S. E., Evtushenko V. V., Lugovsky V. A., et al.* Nitric oxide delivery during cardiopulmonary bypass reduces acute kidney injury: a randomized trial. *J Thorac Cardiovasc Surg*. 2022; 163 (4): 1393–1403.e9. DOI: 10.1016/j.jtcvs.2020. 03.182. PMID: 32718702.
19. *Niebler R. A., Chiang-Ching H., Daley K., Janecke R., Jobe S. M., Mitchell M. E., Varner C., et al.* Nitric oxide added to the sweep gas of the oxygenator during cardiopulmonary bypass in infants: a pilot randomized controlled trial. *Artif Organs*. 2021; 45 (1): 22–28. DOI: 10.1111/aor. 13788. PMID: 32737900.
20. *Schlapbach L. J., Gibbons K. S., Horton S. B., Johnson K., Long D. A., Buckley D. H.F., Erickson S., et al.; NITRIC Study Group, the Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG), and the ANZICS Paediatric Study Group (PSG).* Effect of nitric oxide via cardiopulmonary bypass on ventilator-free days in young children undergoing congenital heart disease surgery: The NITRIC randomized clinical trial. *JAMA*. 2022; 328 (1): 38–47. DOI: 10.1001/jama. 2022.9376. PMID: 35759691.
21. *Lowson S. M., Hassan H. M., Rich G. F.* The effect of nitric oxide on platelets when delivered to the cardiopulmonary bypass circuit. *Anesth Analg*. 1999; 89 (6): 1360–1365. DOI: 10.1097/00000539-199912000-00005. PMID: 10589608.

Received 10.06.2023
Accepted 11.12.2023
Online first 18.12.2023